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Stereochemical specificity of neutral amino acid transfer systems in rat small intestine

It is now well established that there are two carriers involved in the intestinal transfer of neutral amino acids[1–4]. These can conveniently be called the sarcosine carrier and the methionine carrier, since sarcosine is transported mainly by one carrier and methionine by the other. A 'betaine carrier' has been described in the hamster small intestine[5], and, although this has not been shown to be involved in amino acid transfer, it may be equivalent to what we have termed the sarcosine carrier. The specificity of these carriers in relation to length of carbon chain and position of the amino group has recently been discussed[6], and the present work explores the stereochemical specificity.

Experiments were carried out with sacs of everted rat intestine prepared from middle fifth of the combined jejunum and ileum. The sacs contained 1 ml of bicarbonate saline[7] and were suspended in 25 ml of bicarbonate saline containing 28 mM glucose and 1 mM [*Me*-^{14}C]methionine or [*carboxy*-^{14}C]sarcosine. The transfer of methionine and of sarcosine was studied in the presence of a number of other amino acids and related substances.

The results are shown in Table I. In the case of those amino acids which inhibit methionine transfer, the L-enantiomorphs inhibit considerably more than the D-enantiomorphs, in confirmation of previous work[8–11]. In contrast, the L- and D-enantiomorphs have nearly equal effects on sarcosine transfer. In particular D-alanine, D-proline and hydroxy-D-proline inhibit sarcosine at least as much as do the corresponding L-enantiomorphs. It is also seen that both enantiomorphs of azetidine (with a four-membered ring) inhibit sarcosine transfer, but pipecolic acid (with a six-membered ring) has less effect. In this respect it is of interest that L-azetidine, but not pipecolic acid inhibits incorporation of proline during protein synthesis in *Escherichia coli* (ref. 12). However, other workers[13] have observed a large inhibition of sarcosine by DL-pipecolic acid in hamster intestine. These authors also stated that L-proline transfer is not

TABLE I

INHIBITION BY L- AND D-AMINO ACIDS AND RELATED SUBSTANCES ON INTESTINAL TRANSFER OF 1 mM SARCOSINE AND 1 mM L-METHIONINE

The substances used in a concentration of 10 mM are shown in the first column.

Inhibiting substance	*Sarcosine*			*L-Methionine*		
	Transfer (μmoles/sac per 30 min)	*Inhibition (%)*	*Number of expts.*	*Transfer (μmoles/sac per 30 min)*	*Inhibition (%)*	*Number of expts.*
None	5.1	—	20	14.3	—	10
L-α-Alanine	3.7	27	17	9.3	35	10
D-α-Alanine	3.4	33	7	13.6	5	4
L-Proline	2.8	45	7	12.9	10	4
D-Proline	2.4	53	5	13.5	6	4
Hydroxy-L-proline	3.8	25	4	12.8	10	4
Hydroxy-D-proline	3.1	39	4	13.2	8	4
L-Cysteine	4.2	18	7	6.1	57	5
D-Cysteine	4.3	16	6	13.6	5	3
L-Serine	4.6	10	7	9.0	37	7
D-Serine	4.3	16	5	12.5	13	6
L-Norleucine	4.7	8	11	3.2	78	5
D-Norleucine	4.5	12	5	12.0	16	3
L-Azetidine	3.0	41	3			
D-Azetidine	3.0	41	3			
L-Pipecolic acid	4.2	18	3			
D-Pipecolic acid	4.2	18	2			

affected by the presence of D-proline, but this could well be a species difference.

Since sarcosine is inhibited as much by D-enantiomorphs as by L-enantiomorphs, kinetic studies were made to dermine quantitatively the relative affinities of L- and D-proline for the sarcosine carrier. Transport of sarcosine from concentrations of 1, 3, 5, 7.5, 10 and 15 mM was measured, and the experiments were repeated in the presence of 10 mM D-proline or 10 mM L-proline. Analysis of the results by Lineweaver–Burk plots gave a K_m value of 13.3 mM for sarcosine and K_i values of 12.2 mM for L-proline and of 10.2 mM for D-proline.

Since L-proline, rather than sarcosine, probably uses the carrier physiologically, the kinetics of L-proline transport by the sarcosine carrier was studied. L-Proline is also handled by the methionine carrier, and it is therefore necessary to block the methionine carrier by L-methionine and then to study the affinity of L-proline for the sarcosine carrier. In the same way, the K_i of D-proline on L-proline was determined in the presence of L-methionine. The K_m of L-proline for the sarcosine carrier was found to be 13.3 mM, and the K_i of D-proline on L-proline transfer by the sarcosine carrier was 10.8 mM. The limitations and errors of K_m determinations in these conditions are appreciated, but the results do suggest that the descending order of the affinity of the

sarcosine carrier is D-proline, L-proline and sarcosine. They certainly show that D-proline is at least as effective as L-proline in inhibiting sarcosine transfer.

This appears to be the first time that a carrier for amino acids has been described in the intestine which has as great an affinity for the D-enantiomorphs as for the L-enantiomorphs. The historical perspective of stereochemical specificity in intestinal transport of amino acids is now interesting. GIBSON AND WISEMAN[14] first showed clearly a definite preference for the L-enantiomorphs, and LIN AND WILSON[15] concluded that the L-configuration was an unambiguous requirement. JERVIS AND SMYTH[16] showed D-methionine could be actively transferred, although its affinity was much less than L-methionine, and other workers have since shown that some other D-amino acids can use the carrier. NEWEY AND SMYTH[3] showed that there were two carriers involved in neutral amino acid transfer, and the stage has now been reached where one of the carriers, the methionine carrier, has a preference (although not an absolute one) for L-enantiomorphs, whilst the other (the sarcosine carrier) may have no stereochemical specificity.

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